POLD1 and *POLE*: preliminary data from a laboratory-based multi-gene panel testing cohort

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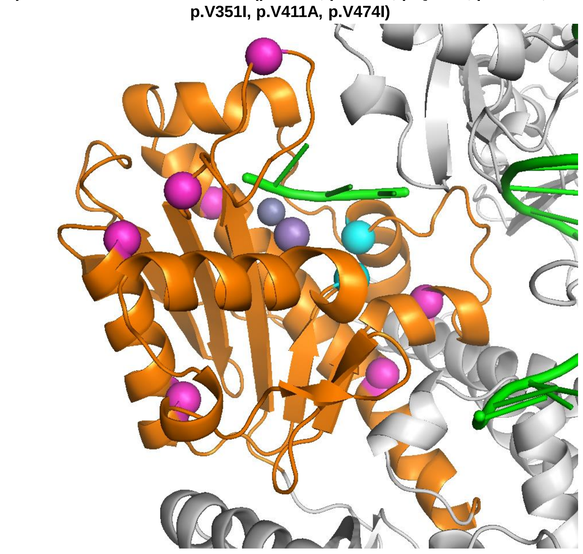
BACKGROUND

- *POLD1* and *POLE are* candidate cancer predisposition genes with clinical and co-segregation data supporting a dominantly-inherited, highly-penetrant colorectal phenotype with extra-colonic tumors also suggested.
- Putative pathogenic mutations have been identified in high-risk colorectal cancer (CRC) and polyposis families.[1-3]
- Publications have focused specifically on single amino acid substitutions impacting highly-conserved residues within the proofreading exonuclease domains of *POLD1* and *POLE*
- *O*nly rare reports of loss-of-function/null (LOF) alleles exist.[4]

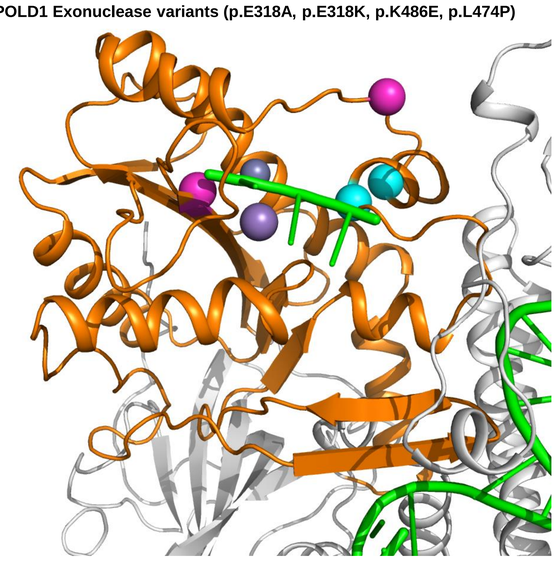
Figure 1: Structural mapping of select POLD1 and POLE missense variants

A) POLE Exonuclease variants (p.E340V, p.P282S, p.Q292H, p.T457M, p.V351I, p.V411A, p.V474I)



Suspected pathogenic variants occur throughout the modelled exonuclease domain of POLE. Known pathogenic variants are located at the DNA binding surface.

B) POLD1 Exonuclease variants (p.E318A, p.E318K, p.K486E, p.L474P)



Suspected pathogenic variants occur at the single stranded DNA or metal ion binding site within the modelled exonuclease domain of POLD1. Known pathogenic variants are located at or near the single stranded DNA binding surface.

Color Key:
Orange Cartoon: Exonuclease protein functional domain
Green Cartoon: DNA
Gray sphere: Metal ion
Teal sphere: Published pathogenic missense mutations
Magenta sphere: Selected missense variants identified in this study

Templates for the modelled structures are from Protein Data Bank ID: POLD1: 3IAY, 1NOY and POLE: 4M80 and 1NOY.

Table 1: Phenotype Summaries- Exonuclease Missense and Loss-of-Function Alleles

A)

Variant	High Sub Pop (denom)	Rare?	In Silico	Phx Adenomas (#)	Phx CRC (Age)	Fhx Polyps (# 1st-2nd rel)	Fhx CRC (# 1st-2nd rel)	Mut Co-Occurrence
POLD1 p.A354V	0.16	N	B	Y (2-5)	N	1	3	
POLD1 p.D402N	absent	Y	D	N	Y (26)	N	2	*PMS2*
POLD1 p.D502G	absent	Y	B	N	N	N	N	
POLD1 p.E318A	absent	Y	D	N	Y (24)	N	2	
POLD1 p.E318K	absent	Y	D	Y (20-99)	N	N	N	*ATM*
POLD1 p.G321S	0.36	N	D	N	N	N	N	
POLD1 p.G321S	0.36	N	D	N	N	N	N	
POLD1 p.G321S	0.36	N	D	N	N	N	1	
POLD1 p.G321S	0.36	N	D	N	N	N	2	
POLD1 p.G321S	0.36	N	D	Y (6-9)	Y (28)	N	1	
POLD1 p.G321S	0.36	N	D	N	N	N	N	
POLD1 p.K486E	absent	Y	D	N	Y (30)	N	N	
POLD1 p.L474P	absent	Y	D	Y (2-5)	N	N	2	
POLD1 p.N430S	absent	Y	B	N	N	N	N	
POLD1 p.P347L	absent	Y	B	N	N	N	3	
POLD1 p.P390S	absent	Y	D	N	N	N	N	
POLD1 p.R311C	0.013	N	D	N	N	N	N	*ATM*
POLD1 p.R311H	absent	Y	D	N	N	N	N	
POLD1 p.R311H	absent	Y	D	N	N	N	N	
POLD1 p.R331Q	0.025	N	B	N	N	N	1	
POLD1 p.R386C	0.018	N	D	N	N	N	1	*APC* (moderate risk)
POLD1 p.R521Q	0.09	N	C	N	N	N	3	
POLD1 p.R521Q	0.09	N	C	N	N	N	N	
POLD1 p.R521W	absent	Y	D	N	N	N	1	
POLD1 p.R525Q	0.02	N	C	N	N	N	N	
POLD1 p.V455M	absent	Y	C	N	N	N	N	
POLD1 p.V489M	absent	Y	D	N	N	n/a	n/a	
POLD1 p.V489M	absent	Y	D	N	N	N	N	
POLE p.A426V	0.01	N	D	N	N	N	N	
POLE p.A427V	absent	Y	B	N	N	N	N	
POLE p.A430T	0.39	N	D	N	N	N	N	
POLE p.A430T	0.39	N	D	N	N	N	1	
POLE p.D287E	0.15	N	D	N	N	N	N	
POLE p.D287E	0.15	N	D	Y (NOS)	N	N	3	
POLE p.D301G	absent	Y	D	N	N	N	N	
POLE p.D339V	absent	Y	D	N	N	N	N	
POLE p.E340V	absent	Y	D	N	Y (64)	N	5	
POLE p.E353D	absent	Y	B	N	N	N	1	
POLE p.H379Y	absent	Y	B	N	N	N	N	
POLE p.P282S	0.015	N	D	Y (NOS)	N	N	2	
POLE p.Q292H	absent	Y	B	N	Y (40)	N	2	
POLE p.Q453E	absent	Y	B	N	N	N	N	*MUTYH (monoallelic)*
POLE p.Q453E	absent	Y	B	N	N	N	N	
POLE p.R446Q	0.041	N	D	N	N	N	N	
POLE p.R446Q	0.041	N	D	N	N	N	N	
POLE p.R446Q	0.041	N	D	N	N	N	N	
POLE p.R446Q	0.041	N	D	N	N	N	N	
POLE p.R446Q	0.041	N	D	N	N	N	N	
POLE p.R446Q	0.041	N	D	Y (10-19)	N	N	N	
POLE p.R446Q	0.041	N	D	N	N	N	N	*BRCA2*
POLE p.S314A	absent	Y	B	N	N	N	1	
POLE p.S314A	absent	Y	B	N	N	N	N	
POLE p.S314A	absent	Y	B	N	N	N	N	
POLE p.S314A	absent	Y	B	N	N	N	N	
POLE p.T457M	absent	Y	D	Y (2-5)	Y (42)	N	1	
POLE p.V351I	absent	Y	B	Y (NOS)	N	N	N	*ATM*
POLE p.V411A	absent	Y	D	Y (20-99)	N	N	N	
POLE p.V437M	absent	Y	D	N	N	N	N	
POLE p.V474I	absent	Y	C	N	Y (35)	N	N	

B)

Variant	Phx Adenomas (#)	Phx CRC (Age)	Fhx Polyps (# 1st-2nd rel)	Fhx CRC (# 1st-2nd rel)	Mut Co-Occurrence
POLD1 c.2251-1G>C	N	N	N	N	
POLD1 p.Q165*	Y (1)	N	Y (1)	N	
POLD1 p.R195*	N	N	N	N	
POLE c.6651_6657+6del13insTGC	N	N	N	N	
POLE p.M1?	N	Y (50s)	Y (1)	Y (3)	*CHEK2*
POLE p.W1980*	Y (10-19)	N	N	N	
POLD1 c.2035_2036delGA	N	N	N	N	
POLE c.2091delC	N	Y (50)	N	N	
POLE c.4290+1G>A	Y (10-19)	Y (68)	N	Y (2)	
POLE c.5180_5181delTG	N	N	N	N	
POLE c.6026_6027delAC	N	N	N	N	
POLE c.6541dupG	N	N	N	N	
POLE p.Q2068*	Y (2)	Y (50)	Y (1)	Y (1)	
POLE p.Q641*	Y (1)	N	N	N	
POLE p.R1909*	N	N	N	Y (1)	
POLE p.R249*	Y (2-5)	Y (26)	Y (1)	N	
POLE p.W1271*	N	Y (46)	N	Y (1)	

Abbreviations: Phx= personal history, Fhx= family history, Mut= pathogenic mutation, NOS – not specified
In Silico: PolyPhen and SIFT models were used; D= deleterious, B= benign, C= conflicting
A) Gray cells: recurrent variants; thick cell borders= variants mapped in Figure 1; orange cell: variant previously described as pathogenic in published literature

METHODS

- *POLD1* and *POLE* analyses were included in three clinically-available next generation sequencing multi-gene panel testing (MGPT) at our diagnostic laboratory as of May 18th, 2015.
- Retrospective data review of >7,500 individuals yielded molecular and clinical details for individuals with identified *POLD1* and *POLE* alterations.

RESULTS

- **Previously described missense mutations** (N=1;0.01%):
  - Recurrent *POLD1* p.S478N and *POLE* p.L424V mutations were not detected in >7500 patients analyzed.
  - *POLD1* p.L474P was identified in one proband with a personal history of colorectal adenomatous polyps and a family history of CRC.
- **Other, rare amino-acid substitutions** within the exonuclease domains, not previously described (N=32;0.42%)
  - 13/32 (40.6%) reported personal and/or family history of CRC and/or polyposis not explained by a co-occurring pathogenic finding in another gene (Table 1).
  - Two observed variants (p.E318A and p.E318K) are highly suspect as pathogenic given their structural location coordinating a Metal ion.
- **LOF alleles** (N=17; 0.22%):
  - 9/17 (52.9%) reported personal and/or family history of CRC and/or polyposis not explained by a finding in another gene

TAKE-HOME POINTS

- Based on our data, the *POLD1* p.S478N and *POLE* p.L424V mutations, which are recurrent in published CRC/polyposis cohorts, are rare in patients referred for MGPT.
- The majority of exonuclease domain missense variants in *POLD1* and *POLE* were identified in families with no reported GI phenotype, were present in population-based frequency cohorts, and/or were predicted to be tolerated by *in silico* and/or structural analysis. As such, most of these findings are not suspected to be pathogenic, although some cases of familial CRC/polyps may be explained by other, rare missense variants within the exonuclease domains.
- Structurally, the most suspicious missense variants appear to have a greater impact on DNA/metal binding rather than domain destabilization.
- As nearly half of LOF allele-positive families did not have any CRC or polyps reported, our data support the hypothesis that LOF alleles in these genes do not confer the same magnitude of risk/penetrance as published pathogenic missense mutations. This may be due to a mechanistic difference in *POLD1* and *POLE* pathogenicity compared to classic tumor suppressor genes. These variants should be reported as “unknown significance” until their contribution to disease, if any, is clarified.
- Continued investigation of *POLD1* and *POLE* findings will further clarify the associated phenotypic spectra, penetrance, and mutation detection rate in clinical cohorts.

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